Ultrastructural Effects of Centrifugation on Eyes of a Snail, *Helix aspersa* [1]

BY

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(1 Plate)

INTRODUCTION

TYPE I SENSORY CELLS in the eye of a snail, *Helix aspersa,* contain a large number of uniform, spherical, 800 Å vesicles which have been named photic vesicles (EAKIN & BRANDENBURGER, 1967, 1970; BRANDENBURGER, 1975). The vesicles are believed to transport the photopigment, or a precursor thereof, from the basal regions of the sensory cells, where they are produced by the Golgi apparatus, to the distal ends of the cells where the microvilli (presumed photoreceptors) are situated. An investigation of the effects of centrifugation was undertaken to test the mobility of the photic vesicles and other structures in the eye.

MATERIAL AND METHODS

Eyes from local adult snails (*Helix aspersa,* Müller, 1774)[2] were dissected in cold saline and placed in paraffin molds, fitted into centrifuge tubes, so that the lens would be directed toward the centrifugal pole. Eyes were spun for 30 minutes at 1700 x g (3500 rpm) in an International Clinical centrifuge placed in a cold box to maintain the temperature inside the centrifuge below 25° C or 30 minutes at 0° C in a Beckman Spinco ultracentrifuge at 7600 x g (10 000 rpm) or at 125 000 x g (40 000 rpm). Extirpated control eyes were held in saline for the same time and at the same temperature. All eyes were prepared for electron microscopy (see BRANDENBURGER, 1975, for methods).

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[2] Voucher specimens have been deposited in the Invertebrate Zoology Collections of the California Academy of Sciences in San Francisco. They have been given the I. Z. Cat. #000955.

RESULTS

Eyes centrifuged at 1700 and 7600 x g retained their normal morphology. Those spun at 125 000 x g exhibited elongation, particularly of the lens, along the optic axis. Irregularities in the retinal cup, such as buckling, were frequently observed.

Sensory cell type I: (type II does not contain photic vesicles, BRANDENBURGER, 1975). Eyes centrifuged for 30 minutes at 1700 x g showed no discernible displacement of cytoplasmic organelles and inclusions. In both experimental and control eyes the photic vesicles occurred in large aggregations, together with beta particles of glycogen and an occasional mitochondrion, near the nuclei of the cells. Small packets of vesicles were found scattered throughout the narrow sensory cell columns—the region of the cell where there is lateral interdigitation with pigmented supportive cells. Very few photic vesicles were observed at the distal (microvillar) ends of the cells.

After centrifugation of eyes at 7600 x g for 30 minutes the masses of photic vesicles still remained intact, and an apical movement of them was not observed. There was a noticeable displacement, however, of mitochondria and endoplasmic reticulum into the apical regions of the sensory cells.

Breakup of the masses of photic vesicles was apparent in eyes centrifuged at 125 000 x g for 30 minutes. The extent of disruption of the vesicles varied from one sensory cell to another. Most sensory cells contained membrane-bounded clusters of vesicles tightly aggregated into a paracrystalline pattern. Other groups of vesicles had fused to form whorls of membranes, and still others had broken down completely into particulate matter (Figure *1*). In some specimens in which the sensory cell columns were not blocked by interdigitating supportive cells large

groups of photic vesicles were found distally (Figure 2) instead of in their normal perinuclear position. Mitochondria and endoplasmic reticulum also showed a distal displacement when centrifuged at this force.

Supportive cells: In control eyes and those spun at 1700 x g for 30 minutes the supportive cells appeared normal (Figure 3). After centrifugation at 7600 x g for the same time, however, their pigment granules were moved to the distal halves of the cells, resulting in broadening the cells in that region but narrowing them basally. The displacement of the pigment and the broadening of the cells distally resulted in the occlusion of many sensory cell columns. The same changes were more pronounced in eyes centrifuged at 125 000 x g (Figure 4). But even at that force the retinal cells did not separate from one another. Desmosomes, hemidesmosomes, septate junctions, and bundles of tonofilaments within the supportive cells appeared normal.

DISCUSSION

Eakin & Brandenburger (1972) have postulated that the photic vesicles are moved from the perinuclear synthetic centers to the microvilli by light-induced, rhythmic contractions of the muscle-containing capsule of the eye of *Helix aspersa*. Centrifugal force, however, does not appear to duplicate the natural massaging effect which they believed the pulsations would effect upon the photoreceptoral cells. At the lowest force used in our experiments, 1700 x g, there was no discernible dislocation of any cytoplasmic organelle. In eyes spun at 7600 x g mitochondria and endoplasmic reticulum were displaced distally, but the masses of photic vesicles in the somas of the cells were still undisturbed. Only at the highest centrifugal force employed, 125 000 x g, were the vesicles moved toward the microvilli, and then in only those receptoral cells with broad columns (*i.e.*, sensory cells with little lateral interdigitation by neighboring supportive cells). The reason why more receptoral cells did not show displacement of the vesicles when spun at 125 000 x g was clearly owing to the movement of pigment granules in the supportive cells which compressed the sensory cell columns so that organelles and inclusions in the receptoral cells could not be displaced distally. We conclude that the normal capsular contractions probably move the vesicles and other cytoplasmic materials very slowly toward the microvilli (in keeping with the general behavior of snails!).

Although we did not establish the easy movability of the photic vesicles by our experiments we learned some other lessons. In many cells the tight aggregation of the photic vesicles increased the number of regions showing paracrystalline patterns. This result supports earlier speculation that the paracrystalline organization is simply the result of close packing of the vesicles. And we have learned that the photic vesicles are fragile and subject to breakdown by pressure. In many cells spun at 125 000 x g whole aggregations of vesicles appeared converted to whorls of membranes that remind us of similar structures in the eyes of snails maintained in total darkness for several months (Eakin & Brandenburger, 1974). Those membranous whorls were believed to arise from a breakdown of photic vesicles.

Lastly, we discovered that the eye of *Helix aspersa* maintained its integrity despite strong centrifugation. The lens became elongated, of course, being a soft gel. And although there was some buckling of the retina at the highest force used, there was no separation of cells. The resistance to deformation was probably owing to the strong desmosomes and septate junctions distally, firm attachments of the supportive cells to the collagenous capsule of the eye by hemidesmosomes, and thick bundles of tonofilaments which run like guys lengthwise through the pigmented cells. The adaptive value of these structural features is easily appreciated by watching under a dissecting microscope a snail extend an optic tentacle by hydrostatic pressure or withdraw it into the head by strong contractions of a retractor muscle. During these move-

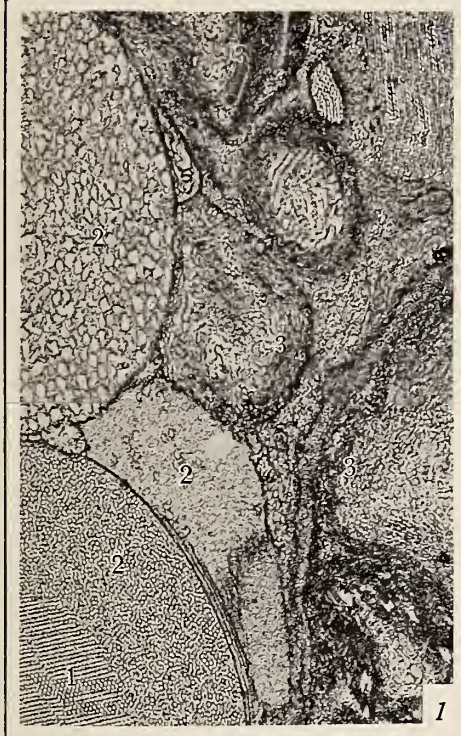
Explanation of Figures 1 to 4

Figure 1: Photic vesicles packed to form paracrystalline patterns (1); vesicles in different stages of breakdown (2); whorls of membranes believed to come from vesicles (3). Centrifuged at 125 000 x g. × 9 000

Figure 2: Sensory cell showing distal displacement of photic vesicles (PV); mitochondria (M); and endoplasmic reticulum (ER). MV, microvilli; SJ, septate junction; Z, adhering zonule. Centrifuged at 125 000 x g. × 7 000

Figure 3: Parts of two pigmented supportive cells bordering a sensory column in an uncentrifuged eye. D, distal microvillar ends of cells; PV, photic vesicles. × 7 000

Figure 4: Dislocation of pigment granules to distal halves of supportive cells and narrowing of sensory columns in eye centrifuged at 125 000 x g. D, distal ends of cells; PV, photic vesicles; TF, tonofilaments. Arrows indicate narrow basal part of a supportive cell. × 7 000



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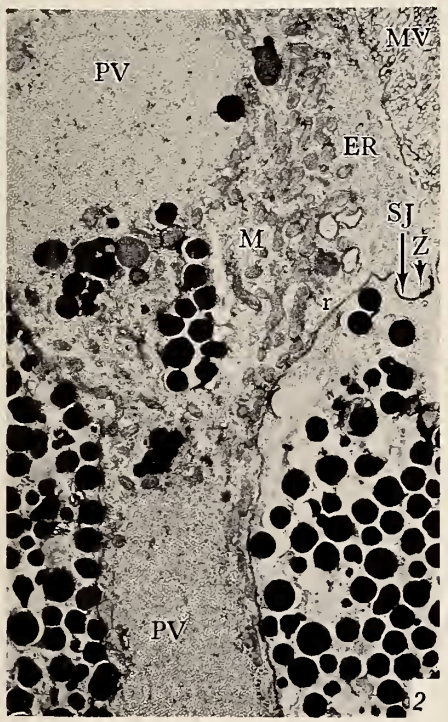

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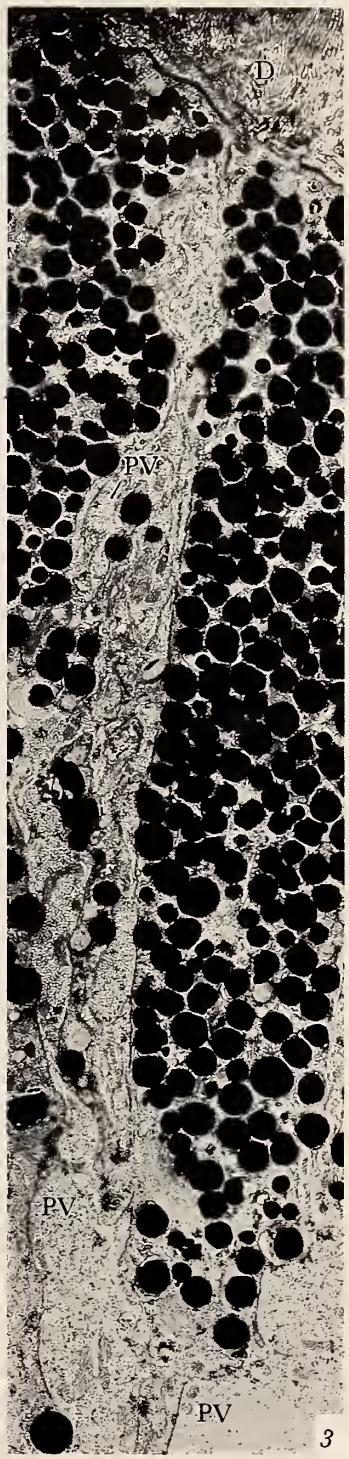

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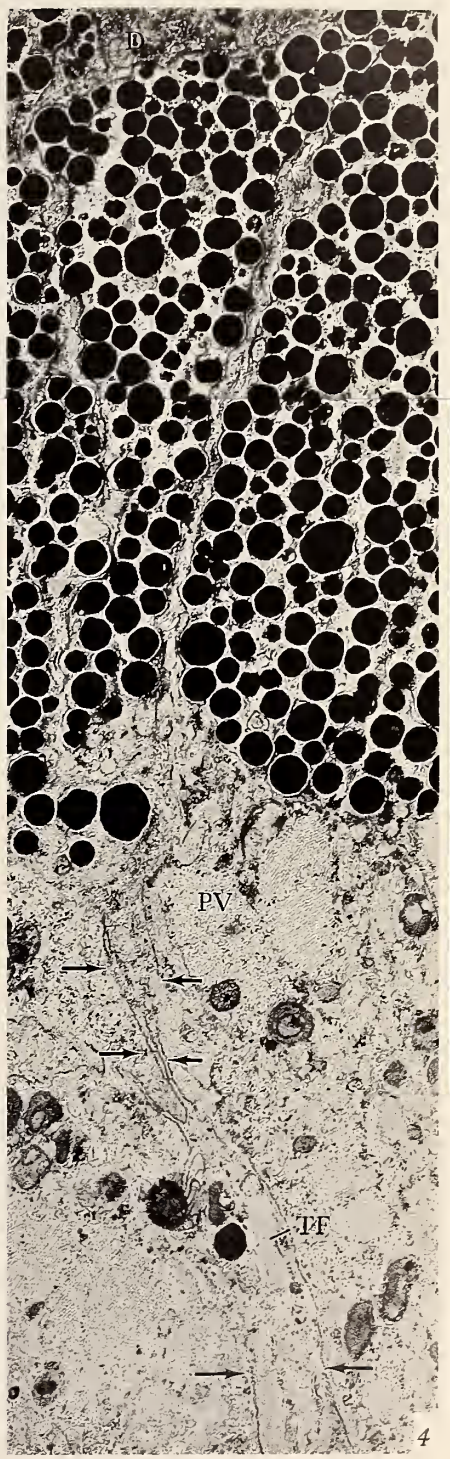

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